BIOLOGICAL PRODUCTS (BIOPHARMACEUTICALS)

10/11/2022

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Outline

- Introduction to biological products
- Proteins and peptides
- Challenges of Recombinant proteins
- Formulation and Delivery Approaches to overcome those challenges
- Therapeutic Biologicals

Biological Products…

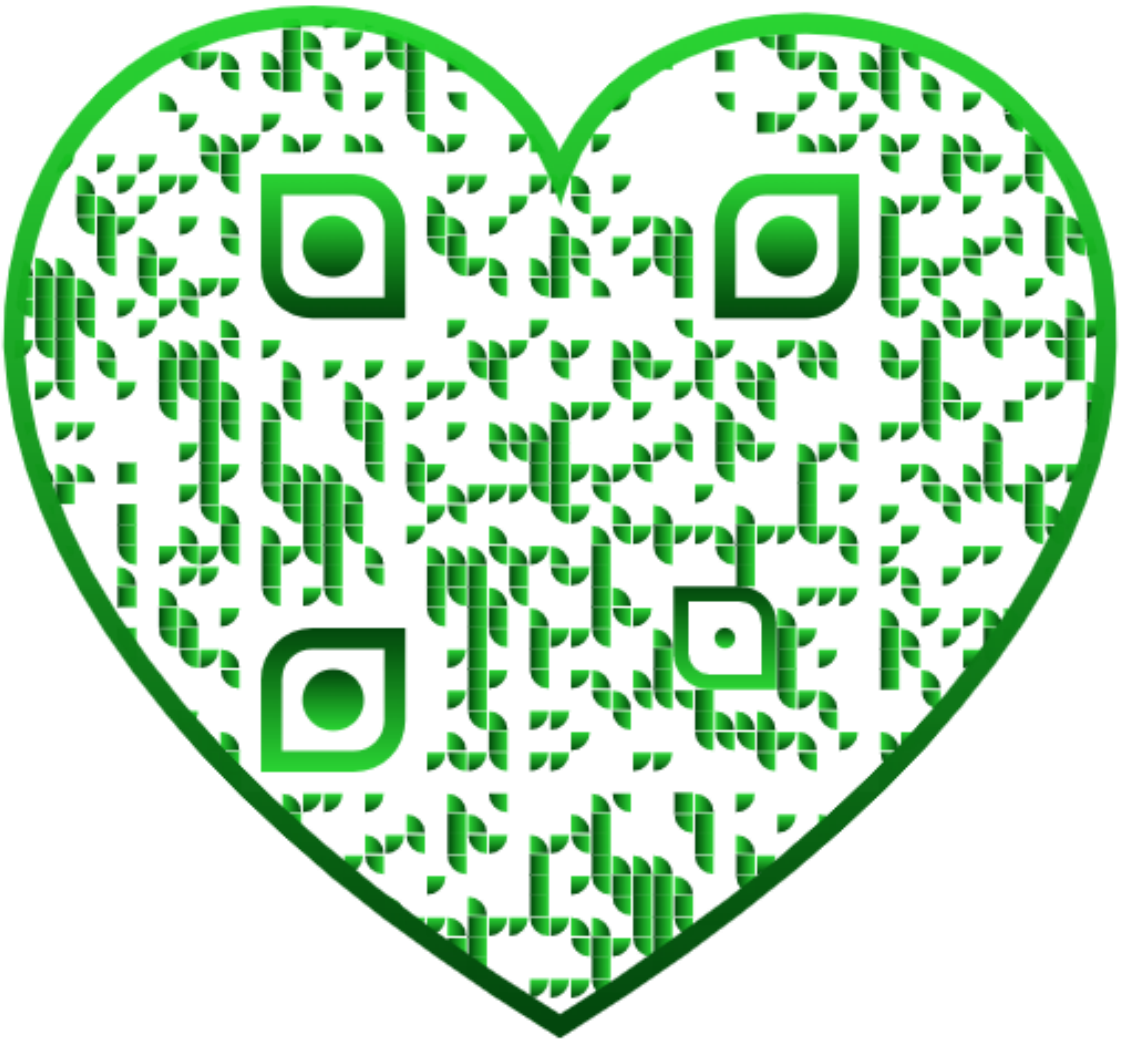
Biologic product:

- Is any virus, therapeutic serum, antitoxin, or analogous product employed for prevention, treatment, or cure of diseases in humans.
  - ✓*structural and mechanistic similarity to naturally occurring substances* in the human body

Biopharmaceuticals versus conventional chemical drugs

- Biopharmaceuticals are different from the conventional chemical drugs by:

1. Their average size.
   - The chemically synthesized products are known as small molecule drugs (e.g. aspirin, molecular weight 180 Da).
   - In general, the biopharmaceuticals are complex macromolecules that are over **100 times** larger (e.g. interferon beta, molecular weight 19,000 Da) with complex structural and appropriate biological activity requirements



Biopharmaceuticals Vs …

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2. Biopharmaceuticals have more potential heterogeneity than small molecule drugs.=> requires regrious purification technique.

- Majority of biopharmaceutical products are derived from life forms Vs Small molecule drugs are not typically regarded as biopharmaceutical in nature by the industry.

3. The nature of the manufacturing process, and the safety and efficacy profile of biopharmaceutical products are also different. => shorter half life, immunogenic, easily degrade by extreme environmental conditions…

Types of biological products

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Biological products have been predominantly **proteins/peptides**

- Hormones
- Monoclonal antibodies
- Enzymes
- Interferons
- Growth factors
- Blood factors
- Interleukins

Types of biological products…

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New biologicals have expanded to include:

- DNA/RNA derivatives
- Tissue or cell therapies- transplantation

2. Proteins and Peptides

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- Proteins are macromolecules made up of simple organic molecules called **amino acids**
- Peptides and proteins only differ by the length of their amino acid chain.
- The shortest peptide is dipeptide….. (<50a.a).
  - **Proteins are polypeptides**
- Each polypeptide consists of a chain of amino acids linked together by **peptide (amide) bonds**

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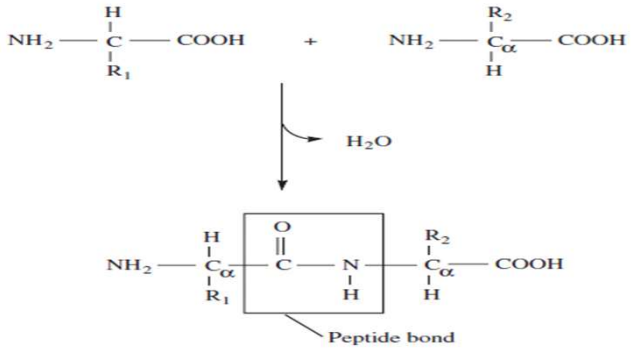

Classification of proteins

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1. On the basis of their overall structure, proteins can be
   - Globular
   - Fibrous

i. **Globular proteins**

- Have compactly folded structures and tend to resemble globes or spheroids in overall shape.
- Some are soluble in water
- Can function in the cytosol or in extracellular fluids
- Others are closely associated with lipid bilayers

Classification of proteins…

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Includes:

- Enzymes
- Antibodies

ii. Fibrous proteins

- They have extended conformations often forming rodlike structures or filaments.
- Are insoluble in water
- A structural or mechanical role--- support or in contraction and relaxation

Classification of proteins…

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Includes:

- Keratin……skin and hair
- Collagen……connective tissue
- Fibroin……silk

Classification of proteins…

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2. Based on their biological function

i. Structural support and connection
- Collagen, Elastin

ii. Catalysis
- Enzymes

iii. Communication
- Hormones, hormone receptors

iii. Defense
- Antibodies

Classification of proteins…

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iv. Transport
- Hemoglobin(O2), Albumin (bilirubin, Ca2+), ion "pumps"

v. Mechanical work- shorten and extend
- Actin, myosin, tubulin

Classification of proteins…

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3. Proteins are sometimes classified as:
- Simple
- Conjugated

i. **Simple proteins**

✓ Consist exclusively of polypeptide chain(s)

ii. Conjugated proteins

✓ Non-polypeptide constituents known as prosthetic group(s):
- Carbohydrates (glycoproteins)
- Phosphate groups (phosphoproteins)
- Vitamin derivatives (e.g. flavoproteins)
- Metal ions (metalloproteins)

Protein post-translational modification

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- After translation, the protein is covalently modified by a variety of small molecules
  - "**Protein posttranslational modification**"=> PTM
    - Affects **biological activity** or the **structural stability** of the polypeptide

Protein PTM…

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The common PTMs include:

- Glycosylation (the most common)
- Phosphorylation
- Carboxylation
- Hydroxylation
- Sulfation
- Amidation

Protein PTM…

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i. Glycosylation

- The addition of one or more sugar molecules
  - **N-linked glycosylation**
  - **O-linked glycosylation**

Protein PTM…

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- **N-linked glycosylation**
  - The sugar chain (the oligosaccharide) is attached to the protein via the **nitrogen atom of** an asparagine (Asn) residue
- **O-linked glycosylation**
  - The sugar chain is attached to the **oxygen atom** of hydroxyl groups, usually those of serine or theronine residues

Protein PTM…

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The potential **roles and effects** of the glycocomponent of glycoproteins

Role/effect	Comment
Protein folding	Glycosylation can effect local protein secondary structure and help direct folding of the polypeptide chain
Protein targeting/trafficking	The glycocomponent can participate in the sorting/directing of a protein to its final destination
Ligand recognition/binding	The carbohydrate content of antibodies, for example, plays a role in antibody binding to monocyte

=> Like pipe leading polypeptide chain

=> Treat infn

Protein PTM…

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Regulates protein half-life	High levels of sialic acid (a family of acidic sugars that often caps sugar side chains) can increase a glycoprotein's plasma half-life. Exposure of galactose residues can decrease plasma half-life by promoting uptake via hepatic galactose residues. Yeast glycosylation is of a 'high mannose' type, which can also drive rapid removal from circulation via specific cell-surface mannose receptors
Immunogenicity	Some glycosylation motifs characteristic of plant-derived glycoproteins (often containing fucose and xylose residues) are highly immunogenic in mammals

Categories of Recombinant proteins

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- Recombinant blood factors
- Recombinant thrombolytics and anticoagulants
- Recombinant hormones
  - Insulin and growth hormones
- Recombinant growth factors
  - Erythropoietin
- Recombinant interferon's and interleukins

Challenges of Recombinant proteins

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- The protein therapeutics ***have unique handling and development requirements:***
  - Stability (in vivo and in vitro)
  - Immune toxicity
  - Pharmacokinetics / pharmacodynamics-related problems

1. Stability

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- **Proteins are susceptible to physical** and **chemical instabilities**

i. **Chemical instabilities**

- Refers to **covalent modifications** of the molecule that result in a new chemical entity
  - Some amino acids in the protein are chemically labile and undergo degradation

1. Stability…

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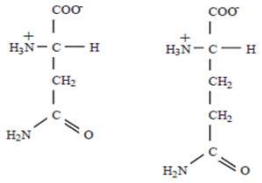
- ***Deamidation***
  - The side chain of amide linkage in a Asparagine or Glutamine residue is hydrolysed to form a fre carboxylic acid



Asparagine Glutamine

1. Stability…

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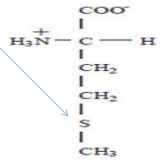
- ***Oxidation***
  - Methionine has been identified as one of the most easily oxidized amino acids in proteins
  - The sulfur atom is susceptible to oxidation.



Methionine

1. Stability…

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ii. Physical Instability

- Physical stability refers to changes in the higher-order structure (secondary and above).
- Manifested as aggregation, precipitation, and surface adsorption

1. Stability…

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1. Denaturation

- *Denaturation refers to an **alteration in the fold** of the* molecule (i.e, a disruption in tertiary and frequently secondary).
- Can be catalyzed by environmental conditions
  - Temperature
  - pH
  - Pressure
  - Denaturing agents such as surfactants- chemicals

1. Stability…

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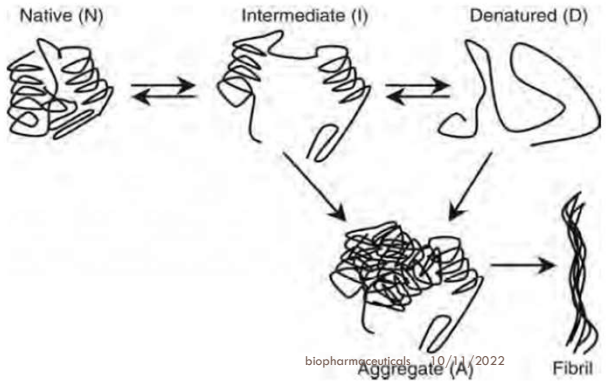

1. Stability…

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2. Aggregation

- Association of protein molecule
- Retains secondary structure but tertiary structural are lost
- Affect the biological activity- **efficacy**

3. Precipitation

- The formation of visible particles
  - Reduce potency
  - Alter the appearance of the formulation

1. Stability…

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4. Surface Adsorption

- Refers to the adsorption of protein on the surface of the immediate containers or filter

Challenges of Recombinant proteins…
2. Immunogenicity of Proteins

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- Proteins and peptides are more immunogenic. and they are more prone to evoke immunotoxicity reactions.
- Epitopes (the part of an antigen molecule to which an antibody attaches itself) are most easily recognized by the immune system. and can evoke a specific immune response
- *Strategy should be designed to reduce immunogenicity*

3. Pharmacokinetic Properties

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- Poor bioavailability
- Rapid elimination from the systemic circulation
- Lack of protein stability within the GIT
  - **Needs technical intervention**

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Formulation and Delivery Approaches to overcome Stability, Immunogenicity & PK Issues

Formulation and Delivery Approaches

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i. Solid formulation- are less prone to hydrolysis

- In solid state proteins are more stable
- Frequently used methods are:
  - Freeze-drying/ lyophilization
  - Spray drying

Formulation and Delivery Approaches…

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ii. Advanced delivery systems

- Pulmonary Delivery
- Nasal Delivery
- Oral/buccal Delivery
- Transdermal Delivery

Formulation and Delivery Approaches…

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iii. Chemical Modification of Proteins and Peptides

- Synthetic **addition** of specific chemical moieties such as polyethylene glycol (**PEGylation)** or fatty acids **(acylation)** to the protein, and modifications by the addition of structures such as glycans to the protein by the host cell (**glycosylation).**
- **Changes in sequence** to a small number of the amino acids making up the protein,
- **Deletions** of a segment of the amino acids of the protein

Formulation and Delivery Approaches…

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1. PEG ylation

- The attachment of a poly(ethylene glycol) (PEG) moiety to the surface of the protein or peptide .

- The benefits of **PEGylation** includes:

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i) improved pharmacokinetics i.e enhanced solubility, improved stability, sustained absorption, continuous biopharmaceutical action,

ii) increased circulation time i.e. decreased amount of protein required for therapeutic efficacy, decreased dosing frequency due to optimized biodistribution, reduced renal clearance

iii) decreased toxicity, improved safety profile, reduced immunogenicity, reduced proteolysis.

Formulation and Delivery Approaches…

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2. Glycoengineering/ glycosylation

- A molecular engineering approach to link saccharides to proteins of therapeutic interest.

Application

- Increase the *in vivo biological activity*
  - Prolonged half – life
- Reduce immunogenicity
  - Carbohydrate can inhibit the antibody formation by ' **shielding** ' the underlying protein sequences from the immune system

Formulation and Delivery Approaches…

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3. Lipidization of Peptides and Proteins —*FA- increases lipophylicity*

- Lipidization may be obtained by **acylation**
  - Through a stable amide bond

Application

i. Prolong the half – life

ii. Improved Delivery via Non–Parenteral Delivery Routes

- Lipidization generally increases cellular uptake of peptides and proteins
- Lipidization protect peptides against enzymatic degradation

Formulation and Delivery Approaches…

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4. Amino Acid Substitution

- example; insulin analogues Lantus®
  - Asparagine at A21 is replaced with a glycine and
  - Two arginine amino acids are further added to the B - terminus
  - Low solubility at physiological pH

=> It forms a slow–dissolving depot of insulin at the subcutaneous injection site

Formulation and Delivery Approaches…

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- NovoRapid® the proline residue at B28 is replaced with an aspartic acid
  - More rapid onset of action when compared to human insulin

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Therapeutic Biologicals

1. **Insulin preparations**
2. **Growth hormone preparations**
3. **Thyroid preparations**
4. **Blood and blood products**
5. **Tumor markers- diagnostic**

Therapeutic Biologicals

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Insulin:

- Insulin is an anabolic hormone which contains 51 amino acids arranged in two chains (A&B) linked by disulfide bridges.
- Insulin regulates blood glucose level in the body by facilitating the uptake of glucose from blood by cells and hence its storage.

Diabetes Mellitus

- A metabolic disorder characterized by deficiency or decreased responsiveness to insulin results from inadequate or absence of insulin.

Therapeutic Biologicals…

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- Type I Diabetes Mellitus (DM)
- Type II diabetes Mellitus

Type I Diabetes Mellitus (DM):

- Characterized by inadequate or complete absence of insulin.
  - Administration of insulin is essential.

Type II diabetes Mellitus:

- Characterized by unresponsiveness of cells to insulin together with a relative deficiency in insulin secretion.
  - Treatment begins with diet, exercise and weight loss, then with oral hypoglycemics (sometimes insulin).
  - Benefit from insulin therapy to control blood glucose (30% or more).

Therapeutic Biologicals…

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Insulin Secretion and Absorption

- Endogenous insulin is produced, stored and released by the beta cells of pancreas.
- It aggregates in to hexameric form and stored in beta cells of pancreas.
- It is released from pancreas when blood glucose levels are elevated.
- This release occurs in two phases
  - *Prandial Insulin Release*
  - *Basal Insulin Release*

Therapeutic Biologicals…

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i. ***Prandial Insulin Release***

- In response to elevated blood glucose level after a meal.
- It occurs in two phases.
  - *Phase- I-* within seconds of ingestion of food and peaks about 1-2 minutes and lasts about 10 minutes.
  - *Phase- II-* occurs within fifteen minutes of phase I and lasts for one to two hours. This phase is responsible for lowering the post-prandial rise in blood glucose.

Therapeutic Biologicals…

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ii. Basal Insulin Release:

- In response to hepatic glucose output. From FA, CHO, Protein
- It has a relatively continuous release of insulin at low levels.
- It helps maintain the appropriate blood glucose level in response to continuous glucose output.

Therapeutic Biologicals…

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- Exogenous human insulin is typically stable in hexameric form:
- Once injected it dissociates in to dimmers and monomers to be absorbed and become biologically active.
- Absorption time of insulin from subcutaneous fat in to the circulation determines whether it mimics prandial or basal insulin action.

Therapeutic Biologicals…

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Advancement of insulin preparations:

- The initial preparations were derived from Pork and highly associated with problems:
  - Impurities and
  - Batch variability in potency.
- In 19 86 recombinant human insulin emerged and
  - produced by either substituting *alanine* for *threonine* on the porcine insulin sequence
- In 19 96, the first insulin analog product ***insulin lispro*** was introduced.

Therapeutic Biologicals…

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Human Insulin Preparations:

- Short acting preparations
  - Regular Insulin(R)
- Intermediate acting preparations
  - Neutral Protamine Hagedorn (NPH) and Lente (L)
- Long acting
  - Ultra lente (U)

- These preparations differ in their pharmacokinetic profile due to protamine and /or zinc in certain formulations

Therapeutic Biologicals…

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Types of Insulin Preparations

- Rapid acting insulin
- Short acting insulin
- Intermediate acting insulin
- Long acting insulin

- Mixtures of insulin preparations
  - Mixed in a syringe prior to administration or premixed.

Therapeutic Biologicals…

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i. **Rapid acting insulin**

- Insulin analogs that include *insulin lispro* and *insulin aspart*.
- they are available clear colorless solutions stable at a neutral P^H.
- Onset of action is with in 1-20 minutes and peak is reached at about 1 hr with duration of 3-5 hrs.
- Designed to mimic phase 2 prandial insulin release
- Rapid dissociation of hexameric structure in to monomers
- Quicker onset of effect and shorter duration of action than R
- *Patients should eat immediately after they inject.*

Therapeutic Biologicals…

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ii. Short acting insulin

- Regular human insulin (R) is typical short acting insulin
- Stable in hexameric form…. due to the presence of low level of zinc.
- Formulated to mimic postprandial insulin release (Phase 2)
- Its onset of action begins about ½ an hour, peaks in about 2-4 hrs and lasts for 6- 8 hrs.
- May cause a slightly increased rate of hypoglycemia
- When administered SC it should be administered approximately 30 minutes before meals.
- It is the only preparation that may be given as an IM or IV

Therapeutic Biologicals…

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iii. Intermediate acting insulin

- Include NPH (Neutral Protamine Hagedorn) and insulin lente (zinc added)
- They are cloudy in their appearance
- Have delayed type of action due to the added protamine and zinc.
- NPH **is** formulated as a crystalline suspension using protamine to complex with R.
- the R-protamine complex remains in hexameric structure longer than R at the injection site.
- NPH is used to mimic basal insulin release.

Therapeutic Biologicals…

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IV. Long acting

- Include *Insulin Glargine* and *Insulin Detemir* and *ultra lente*.
- They are aimed to mimic basal insulin release.
- They are injected once daily and are with onset of action just over an hour and a sustained release for 24 hours
- Ultralente preparations have a cloudy look with onset of about 2 hrs, peak 6-20 hrs and duration of about 36 hrs.

Therapeutic Biologicals…

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V. Premixed insulin preparations

- Premixed insulin preparations are designed to offer both post prandial and basal glucose control with minimal injections and dose manipulation.
  - Reduced daily injections and
  - Decreased dosing errors and patient acceptability
- Premixing is commonly between intermediate acting and rapid acting insulin preparations.
- Examples: 75 % NPH /25% insulin lispro.

2. Growth hormone Preparations

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- Growth hormone is a peptide hormone produced by anterior pituitary and
- It produces growth at open epiphyses via stimulation of insulin like growth factor I (IGF I).
- Human pituitary growth hormone (Somatotropin) is a 191 amino acid peptide with two sulfhydryl bridges.

i. Somatropin

- It is identical to human growth hormone: it has 191 aa.
- It should be stored in air tight container at a temp range of 2-8°C

2. Growth hormone Preparations…

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ii. Somatrem

- It is a 192 aa hormone with additional methionine residue at the amino terminal end.
- It is produced by rDNA procedure in E.coli.
- It is administered IM or SC.
- Therapy with rGH appears to be effective for infants with intrauterine growth retardation

3. Thyroid prepations

61

- ⇒ involved in normal metabolism, growth, and development;
  - ⇒ promotes gluconeogenesis, increases utilization and mobilization of glycogen stores and
  - ⇒ stimulates protein synthesis, increases basal metabolic rate

Classified in to four groups:

- Crude Hormones prepared from animal thyroid (dessicated thyroid)
- Synthetic levothyroxine (L-thyroxine) sodium
- Synthetic liothyronine (L-triiodothyronine) sodium
- Synthetic combinations of L-thyroxine and L-triiodothyronine

3. Thyroid prepations…

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i. Dessicated thyroid

- Its use is never justified because of disadvantages like protein antigenicity, product instability, variable hormone concentration and difficulty in lab monitoring.
- Its shelf life isn't certainly known and potency is better preserved if it is kept dry.

3. Thyroid prepations…

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ii. Synthetic levothyroxine

- Preparation of choice for thyroid replacement:
  - Due to its stability,
  - content uniformity,
  - low cost,
  - lack of allergic foreign protein,
  - long half life (7 days) and
  - it is easy for monitoring its serum level by laboratory methods.

3. Thyroid prepations…

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iii. Liothyronine

- Three to four times more potent than levothyroxine
- Not recommended for routine replacement therapy
  - Its short half life,
  - Higher cost and
  - Difficulty of monitoring its adequacy of replacement by conventional laboratory tests.

4. Enzymatic Preparations

65

i. **Erythropoietin**

- A protein that enhances erythropoiesis by stimulating the formation of pro-erythroblasts and the release of reticulocytes from bone marrow
- It is secreted by the kidney in response to hypoxia
- It is employed to correct erythropoietin deficiency in anemic patients.
- Kidney disease also impairs body's ability to produce this substance.
  - So the administration of erythropoietin is important in **RF**.

4. Enzymatic Preparations…

66

ii. Epoietin alpha

- A glycoprotein produced by r DNA technology and it contains 165 aa (with erythropoietin).
- Epoietin alpha is approved for use in anemia related to cancer chemotherapy, chronic dialysis and AZT therapy.
- Its administration IV or SC, 50-100 IU/Kg body weight three times per week
- A vial of epoietin alpha should not be shaken because the glycoprotein structure may be denatured
- It should be kept between 2 and 8 °C and freezing should be avoided

4. Blood and Blood Products

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Blood Product :

- Any therapeutic substance prepared from human blood and includes:
  - Whole blood,
  - Blood components and
  - Plasma derivatives

1. Whole Blood

- Contains donor`s blood, anticoagulant-preservative solution, hemoglobin and hematocrit.

4. Blood and Blood Products…

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Whole Blood…

- Given when red cell replacement is needed in acute blood loss with hypovolemia
- ABG and Rh compatibility should be ensured for administration
- Medication should never be added to a whole blood.
- It should be stored between 2 and 6°C in approved blood bank refrigerators.
- Transfusion should be started with in 30 minutes of removal from refrigerator.

4. Blood and Blood Products…

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Limitation:

- Whole blood is capable of transmitting any agent present in cells or plasma
  - Infections like HIV, Hepatitis B and C, other hepatitis viruses, Syphilis, Malaria etc

4. Blood and Blood Products…
2. Blood Components

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- A constituent of blood separated from whole blood, such as *Red cell concentrates*, *Red cell suspensions*, *Plasma* and *Platelet concentrates*

4. Blood and Blood Products…
2. Blood Components…

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A. Red cell concentrates

- Most of the plasma has been removed and contains Hemoglobin approx 20 g/100 ml and Hematocrit 55-75%.
- It is given as a replacement for **red cells** in anemic patients.
- Has the same risks of infection, storage and administration recommendations like that of whole blood.

4. Blood and Blood Products…
2. Blood Components…

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B. Red cell Suspension

- It contains red cells with minimal residual plasma to which normal saline, adenine, glucose mannitol solution (SAG-M) are added.
- The hemoglobin content is approximately 15g/100 ml and hematocrit 50-70 %.
- Given for the same conditions to that of red cell concentrates – replacement of red cell in anemic patient

4. Blood and Blood Products…

2. Blood Components…

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C. Plasma

i. Fresh frozen Plasma

- It is separated from one whole blood donation with in six hours of collection and then rapidly frozen to -25°C or colder.
- It contains normal plasma levels of **stable clotting factors**, albumin and immunoglobulin.
- Given in the replacement of multiple **coagulation factor deficiencies**
- Should be stored at a temperature of -25 or colder
- Administration requires ABO compatible

4. Blood and Blood Products…

2. Blood Components…

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D. Platelet Concentrates

- It should contain at least **55 x 10^9 platelets**, < 1.2 x 10^9 red cells and < 0.12 x 10^9 leucocytes.
- It is given for the treatment of bleeding due to thrombocytopenia, and platelet function defect.
- shouldn't be refrigerated before infusion as this reduces platelet function
- Should be infused over a period of about 30 minutes.
- Platelet concentrates should be ABO compatible

4. Blood and Blood Products…

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3. Plasma Derivatives

- Include Albumin, Coagulation factor concentrates and immunoglobulins.

i. **Albumin**

- It is a highly water soluble protein and an important component of the plasma.
- Albumin functions as a **carrier** for hormones, enzymes, fatty acids, metal ions and medicinal products.
- Used as replacement therapy in trauma, surgery and blood loss burns management and plasma exchange.

4. Blood and Blood Products…

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3. Plasma Derivatives…

ii. Coagulation Factors

A. Factor VIII concentrate

- Given as a treatment for hemophilia A
- It is administered as a dissolved powder in solution form
- The storage temperature is in the range of 2 to 8°C.

4. Blood and Blood Products…

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3. Plasma Derivatives…

iii. Immunoglobulins

Immunoglobulin for IM use:

- It is a concentrated solution of IgG antibody component of the plasma
- It contains antibodies against infectious agents to which the donor population has been exposed.
- Given for the prevention of hemolytic disease of the new born.

5. Tumor Markers…

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ii. Human Chorionic Gonadotropin(HCG)

- a glycoprotein composed of two dissimilar sub units (a & b chains),
- it contains 30% carbohydrate.
- It is the most importantly a marker of germ cell tumors
- The values in serum of healthy men and non pregnant women are less than 5 IU/ml.

5. Tumor Markers- diagnostics

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- Are molecules occurring in blood or tissue associated with cancer.
- It can be produced by either the tumor itself or by the body in response to the tumor.

i. **Alpha-fetoprotein (AFP)**

- Carcino-embryonic glycoprotein /Oncofetal antigen.
- It is single chain glycoprotein with 3% carbohydrate and amino acid sequence similar to albumin.
- It is expressed during malignancy or during intrauterine time
- AFP that exceeds 500ng/ml indicates underlying malignancy except in the case of pregnancies.

5. Tumor Markers…

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iii. Prostatic Specific Antigen (PSA)

- It is a 34 KD single chain glycoprotein and the most useful and clinically relevant tumor markers for carcinoma of prostate.
- It is also excellent marker for monitoring the efficacy of treatment modality.

iv. Calcitonin

- It is a low molecular weight circulating peptide hormone and used as tumor marker because its concentration increases in malignancies with skeletal metastases.

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Nucleic acid based biopharmaceuticals

Nucleic acid based biopharmaceuticals

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- Nucleic-acid-based therapeutics centre around
  - Gene therapy
  - Antisense technology

Nucleic acid based biopharmaceuticals…

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- Correcting genetic defects
- Over expressing proteins that are therapeutically useful, or
- Inhibiting the production of "harmful" proteins

Nucleic acid based biopharmaceuticals

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i. **Gene therapy**
   - The transfer of nucleic acids (DNA or RNA) to somatic cells of a patient, which results in a therapeutic effect.

Nucleic acid based biopharmaceuticals…

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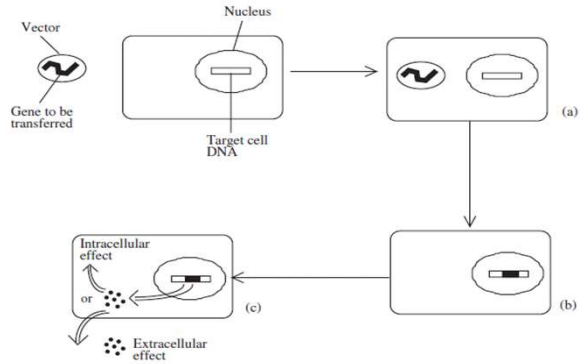

2. Antisense technology

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- Various disease states are associated with the inappropriate production/ overproduction of gene products.
  - The overproduction of angiotensinogen, which ultimately results in hypertension
- Inappropriate gene (over) expression could be prevented if this expression could be **downregulated**
  - A nucleic-acid-based approach to achieve this is termed **'antisense technology**

2. Antisense technology…

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- The antisense approach is based upon the **generation** of **short, single-stranded stretches of nucleic acids** (which can be **DNA- or RNA-based**) displaying a specific nucleotide sequence
- These are generally termed **'antisense oligonucleotides'**

2. Antisense technology…

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- It is possible to synthesize chemically an oligonucleotide whose base sequence is **complementary** to at least a section of the mRNA sequence
  - Antisense oligonucleotide
- The antisense oligonucleotide can hybridize with the mRNA

2. Antisense technology…

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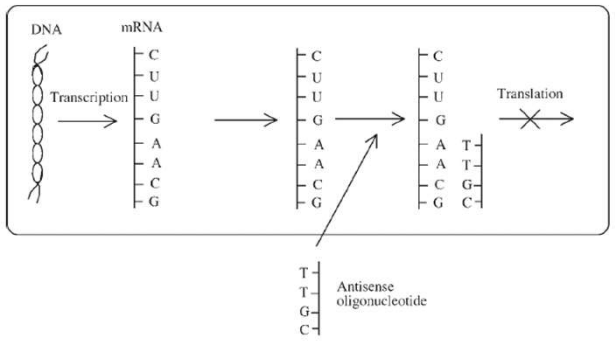

2. Antisense technology…

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- Binding results in the **blocking of translation** of the mRNA and, hence, prevents synthesis of the mature gene's protein product
  - The oligonucleotides act as **steric blockers**

2. Antisense technology…

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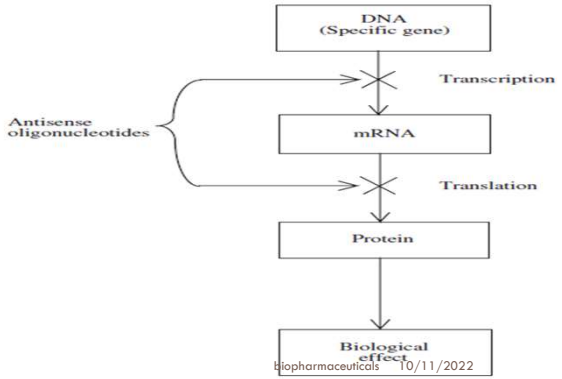

2. Antisense technology…

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Uses

- Cancer
- Viral diseases (e.g. HIV, hepatitis B, herpes and papillomavirus infections)
- Rheumatoid arthritis

2. Antisense technology…

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Advantages of 'oligos'

- *Specificity*
  - Specific base sequence
- *Relatively low toxicity*
  - Specificity
  - Natural biomolecules
- *Requirement for only low levels of the oligo*
- *Manufacruring is straightforward using automated synthesizers*

2. Antisense technology…

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Disadvantages include:

- Sensitivity to nucleases
- Very low serum half lives
- Poor rate of cellular uptake
- Orally inactive

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